

PROMOTER-DEBUGGING MODULE: LAB NOTEBOOK WEEKLY SUMMARY

Week 1 (1/6/2015-5/6/2015)

1. Summer training, mainly for recalling the basic techniques
2. Discussion for the plan of the main project

Week 2 (8/6/2015-12/6/2015)

1. Transformation of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840
2. Inoculation of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840
3. Plasmid extraction of pSB1C3-BBa_I0500, pSB1C3-BBa_B0015, pSB1C3-BBa_B0030, pSB1C3-BBa_K381001 and pSB1C3-BBa_E0840
4. PCR cloning of *phoR* from *E. coli* strain DH10B
5. Phosphorylation and annealing of *phoAp*
6. Digestion of pSB1C3-BBa_J04450
7. Ligation of *phoR* with pSB1C3, *phoAp* with pSB1C3 and pSB1C3-BBa_I0500 with pSB1C3-BBa_B0030
8. Colony PCR for identifying candidate colonies for pSB1C3-*phoR*

Week 3 (15/6/2015-19/6/2015)

1. Transformation of pSB1A2-BBa_B0030, pSB1A2-BBa_E0840, pSB1AK3-BBa_B0015
2. PCR reaction for identifying candidate colonies for pSB1C3-*phoR* and pSB1C3-BBa_I0500-B0030
3. Phosphorylation and annealing of *phoAp*
4. Digestion of pSB1C3-BBa_J04450
5. Sequencing of pSB1C3-*phoR*

Week 4 (22/6/2015-26/6/2015)

1. Colony PCR for checking the identity of pSB1C3-*phoR* candidate 7 and 8 by VF₂ and VR primers

2. Colony PCR for checking the identity of pSB1C3-*phoR* candidate 7 and 8 by F_{phoR} and VR primers
3. Restriction check of pSB1C3-*phoR* candidate 7 and 8 using BstEII
4. Colony PCR for checking the identity of pSB1C3-*phoAp* candidate 1 and 3 by VF₂ and VR primers
5. Restriction check of pSB1C3-*phoAp* candidate 1 and 3 using PvuII
6. PCR cloning of *nsrR* from *E. coli* strain DH10B
7. Ligation of *nsrR* with pSB1C3
8. Transformation of pSB1C3-*nsrR*

Week 5 (29/6/2015-3/7/2015)

1. New plan for the constructs: instead of having constructs of pSB1C3-*nsrR* and pSB1C3-*phoR* for sequencing, rather, ligate *nsrR* and *phoR* with pSB1C3-BBa_B0030, respectively, while no major change for *phoAp*.
2. Digestion of pSB1C3-BBa_B0030.
3. PCR cloning of *nsrR* and *phoR* from *E. coli* strain DH10B.
4. Digestion of PCR product of *nsrR* and *phoR*.
5. Ligation of pSB1C3-BBa_B0030 with *nsrR* and *phoR* respectively.
6. Digestion of pSB1C3-BBa_J04450.
7. Overnight ligation of *phoAp* with pSB1C3 at 16°C.
8. Transformation of pSB1C3-BBa_B0030-*nsrR*, pSB1C3-BBa_B0030-*phoR* and pSB1C3-*phoAp*.
9. Colony PCR for checking the identity of pSB1C3-BBa_B0030-*nsrR*, pSB1C3-BBa_B0030-*phoR* and pSB1C3-*phoAp* candidate colonies. Identified candidate 1 and 5 of pSB1C3-BBa_B0030-*phoR* and candidate 1-17 of pSB1C3-*phoAp*.
10. Confirmed the identity of candidate 1 and 5 of pSB1C3-BBa_B0030-*phoR* by restriction check using PvuII, ready for sequencing.
11. Digestion of pSB1C3-BBa_B0030
12. Ligation of pSB1C3-BBa_B0030 with *nsrR*.
13. Transformation of pSB1C3-BBa_B0030-*nsrR*.
13. New plan for *phoAp*, instead of having the construct of pSB1C3-*phoAp* for sequencing, rather, ligate *phoAp* with pSB1A2-BBa_E0840 for sequencing.

14. Digestion of pSB1A2-BBa_E0840
15. Overnight ligation of pSB1A2-BBa_E0840 with *phoAp* at 16°C.

Week 6 (6/7/2015-10/7/2015)

1. Send pSB1C3-BBa_B0030-*phoR* candidate 5 for sequencing.
2. Ligation of pSB1C3-BBa_B0030 with *nsrR*.
3. Transformation of pSB1C3-BBa_B0030-*nsrR*.
4. Colony PCR of pSB1C3-BBa_B0030-*nsrR* candidate 1-22.
5. Identified candidate 3 and 13 of pSB1C3-BBa_B0030-*nsrR* by colony PCR.
6. Restriction check of pSB1C3-BBa_B0030-*nsrR* candidate 3 and 13 by *ScaI*.
7. Identified pSB1C3-BBa_B0030-*nsrR* candidate 13 by restriction check.
8. Send pSB1C3-BBa_B0030-*nsrR* candidate 13 for sequencing.
9. Transformation of pSB1A2-*phoAp*-BBa_E0840.
10. Identified pSB1A2-*phoAp*-BBa_E0840 candidates by restriction check using *PvuII*.
11. Miniprep of pSB1C3-BBa_B0030-*phoR* candidate 5, 7, 8, pSB1C3-BBa_B0030-*nsrR* candidate 3 and
13, pSB1A2-*phoAp*-BBa_E0840 candidate 1-5.
12. Ligation of pSB1A2-BBa_E0840 with *phoAp*.
13. Send pSB1A2-*phoAp*-BBa_E0840 candidate 5 for sequencing.
14. Sequencing result of pSB1C3-BBa_B0030-*phoR* candidate 5 showed missing of BBa_B0030.
15. Inoculation and streak pSB1C3-BBa_B0030, pSB1AK3-BBa_B0015

Week 7 (13/7/2015-17/7/2015)

1. Send pSB1C3-BBa_B0030, pSB1C3-BBa_B0030-*phoR* candidate 1, pSB1A2-*P_{phoA}*-BBa_E0840 for sequencing.
2. Confirmed pSB1A2-*phoAp*-BBa_E0840 from the sequencing result.
3. From sequencing result of pSB1C3-BBa_B0030, it showed extra nucleotides before and after the BBa_B0030 sequence, which is inconsistent with the sequence from the iGEM Parts Registry.
4. Transformation of pSB1C3-BBa_B0032.

5. Miniprep of pSB1C3-BBa_B0032 and pSB1C3-BBa_B0030.
6. Digestion of pSB1C3-BBa_B0032.
7. Ligation of pSB1C3-BBa_B0032 with *phoR* and *nsrR* separately.
8. Transformation of pSB1C3-BBa_B0032-*nsrR* and pSB1C3-BBa_B0032-*phoR*.
9. Colony PCR of pSB1C3-BBa_B0032-*nsrR* candidate 1-8 and pSB1C3-BBa_B0032-*phoR* candidate 1-8.
10. Restriction check of pSB1C3-BBa_B0032-*nsrR* candidate 5 and pSB1C3-BBa_B0032-*phoR* candidate 6.
11. Glycerol stock for pSB1AK3-BBa_B0015.
12. Functional assay for *phoAp* and *yeaRp*.
12. Characterization of *phoAp* and *yeaRp*.
13. Prepared modified M9 minimal medium for *yeaRp* characterization with NH_4Cl being replaced by MOPS salt for testing the viable range.
14. Prepared modified M9 minimal medium for *P_{phoA}* characterization with phosphate being replaced.

Week 8 (20/7/2015-24/7/2015)

1. Digestion of *nsrR*, *phoR* PCR product and pSB1C3-BBa_I0500-B0030.
2. Ligation of *nsrR* and *phoR* with pSB1C3-BBa_I0500-B0030 separately.
3. Transformation of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR*.
4. Colony PCR for identifying candidates of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR*.
5. Functional assay of pSB1C3-BBa_K381001 with 0mM and 20mM KNO_3 .
6. Characterization of pSB1C3-BBa_K381001 with series of 0mM, 5mM, 10mM, 15mM, 20mM, 50mM, 100mM KNO_3 .
7. Characterization of pSB1C3-BBa_K381001 with series of 0mM, 2mM, 4mM, 6mM, 8mM, 10mM KNO_3 .
8. Ligation of *nsrR* and *phoR* with pSB1C3-BBa_I0500-B0030 separately. (new)
9. Transformation of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR*. (new)
10. Inoculate pSB1A2-*phoAp*-BBa_E0840 in M9 minimal medium with 0 μM , 10 μM , 500 μM and 1000 μM of PO_4 (Using Tris hydrochloride to replace the original PO_4 in M9)

11. Check OD and GFP expression

12. Digestion and gel purification of pSB1A2-BBa_E0840 (for ligating with pSB1A2-BBa_J23101 to act as a positive control)

Week 9 (27/7/2015-31/7/2015)

1. Characterization of pSB1C3-BBa_K381001 in M9 minimal medium with 0μM, 20μM, 200μM, 2000μM KNO₃.

2. Characterization of pSB1C3-BBa_K381001 in LB for 3 trials with 0mM, 2mM, 4mM, 6mM, 8mM, 10mM KNO₃. (Followed directly the protocol from BCCS-Bristol 2010)

As the constructs of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR* were stuck for 2 months in the cloning process, some trouble-shoots were adopted as follows: (point 1-9)

3. PCR cloning of *nsrR* and *phoR* from DH10B, with 4 tubes of 50μl reaction.

4. PCR purification using small column, 5μl after 5μL for eluting the sample.

5. Digestion of *nsrR* and *phoR* with XbaI and PstI-HF digestion enzymes in 37°C incubator for 2 hrs.

6. PCR purification using small column, 5μl after 5μL for eluting the sample.

7. Ligation of *nsrR* with pSB1C3-BBa_I0500-B0030 and *phoR* with pSB1C3-BBa_I0500-B0030 for 3 hrs in 16°C incubator.

8. Transformation of pSB1C3-BBa_I0500-B0030-*nsrR* and pSB1C3-BBa_I0500-B0030-*phoR*.

9. Colony PCR of pSB1C3-BBa_I0500-B0030-*nsrR* candidate 1-26 and pSB1C3-BBa_I0500-B0030-*phoR* candidate 1-26.

10. Characterization of pSB1A2-*phoAp*-BBa_E0840 in M9 minimal medium with 0μM, 30μM, 50μM, 100μM, 150μM, 200μM, 250μM PO₄³⁻.

11. Digestion of pSB1A2-BBa_J23101-J61002 (constitutive promoter-RFP reporter)

12. Ligation of BBa_J23101 (constitutive promoter) and BBa_E0840(GFP reporter)

13. Streak plates for pSB1A2-*phoAp*-BBa_E0840 candidate 5, pSB1A2-E0840 and pSB1A2-BBa_J23101-E0840

Week 10 (3/8/2015-7/8/2015)

1. Restriction check on pSB1C3-BBa_I0500-B0030-*nsrR* candidate 19-23, and 26.

2. Sent pSB1C3-BBa_I0500-B0030-nsrR candidate 23 for sequencing
3. Characterization of pSB1C3-BBa_K381001 using LB medium for trial 4-6 with concentration gradient of 0, 2, 4, 6, 8, 10, 15, 20mM KNO₃
4. Characterization of pSB1C3-BBa_K381001 using M9 minimal medium for trial 1-3 with concentration gradient of 0, 2, 4, 6, 8, 10mM KNO₃
5. PCR cloning of *phoR* from DH10B
6. Digestion of *phoR*.
7. Ligation of *phoR* with pSB1C3-BBa_I0500-B0030 (*P_{bad}*-RBS)
8. Transformation of pSB1C3-BBa_I0500-B0030-*phoR*.
9. Colony PCR of pSB1C3-BBa_I0500-B0030-*phoR*.
10. Characterization of pSB1A2-BBa_*P_{phoA}*-E0840.

Week 11 (10/8/2015- 14/8/2015)

1. Sent pSB1C3-BBa_I0500-B0030-nsrR candidate 23 for sequencing
2. Characterization of pSB1C3-BBa_K381001 using LB medium for trial 8 with concentration gradient of 0, 2, 4, 6, 8, 10, 15, 20mM KNO₃
3. Characterization of pSB1C3-BBa_K381001 using M9 minimal medium for trial 4-6 with concentration gradient of 0, 2, 4, 6, 8, 10mM KNO₃
4. Characterization of pSB1C3-BBa_K381001 using M9 minimal medium for trial 1-5 with concentration gradient of 0, 20, 200, 500, 1000, 2000μM KNO₃
5. Restriction check of pSB1C3-BBa_I0500-B0030-*phoR* candidate 6-10 using enzyme BstEI
6. Sent pSB1C3-BBa_I0500-B0030-*phoR* candidate 9 for sequencing

Week 12 (17/8/2015- 23/8/2015)

1. Ligate pSB1C3-BBa_I0500-B0030-*nsrR* with pSB1C3-BBa_K381001
2. Identifying correct candidates for pSB1C3-BBa_I0500-B0030-*nsrR*-K381001 by restriction check and colony PCR.
3. Characterization of *yeaRp* promoter in M9 minimal medium with concentration of 0-500μM, and in LB with concentration of 0-50mM.
4. Gibson Assembly on pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct.
5. Identifying candidates for pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct using colony PCR.

6. Characterization on *phoAp* promoter.
7. Characterization on *phoBRp* promoter.
8. Characterization on *AMNp* promoter.

Week 13 (24/8/2015-30/8/2015)

1. 1. Ligate pSB1C3-BBa_I0500-B0030-*nsrR* with pSB1C3-BBa_K381001
2. Identifying correct candidates for pSB1C3-BBa_I0500-B0030-*nsrR*-K381001 by restriction check and colony PCR.
3. Confirmed pSB1C3-BBa_I0500-B0030-*nsrR*-K381001 candidate 6 is correct.
3. Characterization of *yeaRp* promoter in M9 minimal medium with concentration of 0-500μM.
4. Gibson Assembly on pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct.
5. Identifying candidates for pSB1C3-BBa_I0500-B0030-*phoR*-B0015 construct using colony PCR and restriction check.
6. Functionality assay on the pSB1C3-BBa_I0500-B0030-*nsrR*-K381001, the debug method with *yeaRp* promoter.
7. Characterization on *phoAp* promoter.
8. Characterization on *phoBRp* promoter.
9. Characterization on *AMNp* promoter.

Week 14 (31/8/2015-6/9/2015)

1. Characterization on the pSB1C3-BBa_I0500-B0030-*nsrR*-K381001, the debug method with *yeaRp* promoter with M9 minimal medium from 0- 2mM nitrate and 10mM arabinose concentration.
2. Characterization on *phoAp* promoter.
3. Characterization on *phoBRp* promoter.
4. Characterization on *AMNp* promoter.

